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(54) **Triglycerides.**

(57) Triglycerides with at least two different acids chosen from 6-desaturated ssential fatty acids and oleic acid, useful in nutrition and in medicine.

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TABLE 1

n-6		n-3
18:2 delta-9,12		18:3 delta-9,12,15
(linoleic acid)	↓	(alpha-linolenic acid)
	delta-6 desaturase	
18:3 delta-6,9,12	↓	18:4 delta-6,9,12,15
(gamma-linolenic acid)		(stearidonic acid)
	elongation	
20:3 delta-8,11,14	↓	20:4 delta-8,11,14,17
(dihomo-gamma-linolenic acid)		
	delta-5 desaturase	
20:4 delta-5,8,11,14	↓	20:5 delta-5,8,11,14,17
(arachidonic acid)		('eicosapentaenoic acid')
	elongation	
22:4 delta-7,10,13,16	↓	22:5 delta-7,10,13,16,19
(adrenic acid)		
	delta-4 desaturase	
22:5 delta-4,7,10,13,16	↓	22:6 delta-4,7,10,13,16,19
		('docosahexaenoic acid')

The acids, which in nature are of the all-cis configuration, are systematically named as derivatives of the corresponding octadecenoic, eicosanoic or docosanoic acids, e.g. delta-9, 12-octadecadienoic acid or delta-4,7,10,13,16, 19-docosahexaenoic acid, but numerical designations such as, correspondingly, 18:2 n-6 or 22:6 n-3 are convenient. Initials, for example, EPA for the 20:5 n-3 acid (eicosapentaenoic acid) or DHA for the 22:6 n-3 acid (docosahexaenoic acid), are also used but do not serve when n-3 and n-6 acids of the same chain length and degree of unsaturation exist as for example with the 22:5 acids. Trivial names in more or less common use in the n-6 series are as shown. Of the n-3 series only 18:3 n-3 has a commonly used trivial name, alphalinolenic acid, though the name stearidonic acid is coming into use for the 18:4 n-3 acid and the names eicosapentaenoic acid and docosahexanenoic acid as such are also used.

Disease States

It is becoming apparent that in many different disease states there are abnormalities of EFA biochemistry leading to abnormal EFA levels in various lipid fractions and in various tissues. These diseases include dis-

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eases of the heart and circulation such as hypertension and coronary and peripheral vascular disease, diseases of inflammation and immunity such as atopic disorders, osteoarthritis, rheumatoid arthritis, ulcerative colitis, Crohn's disease and various disorders going under the general classifications of inflammatory or autoimmune, neurological disorders such as Alzheimer's disease, Parkinson's disease and multiple sclerosis, disorders of the kidney, disorders of the skin, disorders of the gastrointestinal tract, disorders of metabolism of calcium and other minerals, disorders of bone and connective tissue, disorders of the reproductive and endocrine systems, psychiatric disorders including schizophrenia, and disorders of aging.

It used to be thought sufficient, both in nutrition and in therapy of disease, to supply linoleic and alpha-linolenic acids and the body's own metabolism would invariably do the rest. It has now been evident for some time that this is not true. Different diseases have different abnormal patterns of EFAs and because of problems in metabolism these cannot be corrected simply by giving linoleic acid or alpha-linolenic acid. Many examples of this type of situation are given in papers and prior patents by the inventor. Relevant papers include Horrobin D.F. Rev. Contemporary Pharmacotherapy 1990: 1:1-41, Horrobin D.F. Progress Lipid Res 1992: 31: 163-194 and Horrobin D.F. and Manku M.S. pp. 21-53 in "Omega-6 Essential Fatty Acids" Ed. Horrobin, D.F. New York: Wiley-Liss, 1990

It is therefore desirable in some situations to give two or more of the EFAs simultaneously. For this purpose the EFAs may be divided into the following groups:

i) GLA and DGLA
ii) AA and its metabolites adrenic acid and the 22:5n-6 acid
iii) Stearidonic acid (SA) and the 20:4n-3 acid
iv) EPA and its metabolites the 22:5n-3 acid and DHA.

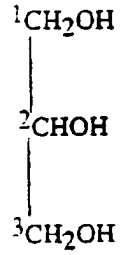
Moreover, the EFAs are exceptionally susceptible to oxidation and so it may be appropriate to co-administer the EFAs with oleic acid (OA) which has potent properties as an antioxidant.

While the EFAs can be supplied in various forms and in various mixtures, it is in principle convenient in both nutrition and in medical treatment to be able to supply the fatty acids as predetermined, particular molecules. This is particularly true with respect to pharmaceuticals, where regulations and directives covering combination products are becoming steadily more restrictive. For example, in order to win government approval for a combination drug product containing compounds A, B and C, it is now no longer adequate to mix the three compounds together in formulation X, and then to compare X with placebo, P. Many governments now require proof of the value of each individual chemical entity, whether or not the whole point of a proposal is a synergistic action of different entities or a newly discovered simultaneous lack of more than one entity. Therefore at the very least clinical studies have to be set up comparing P with X, with A alone, with B alone and with C alone. Some governments might also require comparisons with A + B, A + C and B + C. Thus at least five and possibly eight groups would be required for testing with an enormous escalation of cost. In order to avoid this situation, it would be appropriate instead of having a mixture of A, B and C, to have a single molecule in which A, B and C are found together in the same chemical compound, Y, allowing direct and simple testing of Y against P with only two groups required. For this purpose triglycerides, which can contain three fatty acids, are proposed.

Triglyceride Structures

In triglycerides the above different groups of EFAs and oleic acid may be present in the same molecule, either randomly distributed among the 1, 2 and 3 positions or with a particular EFA being found specifically in one of the positions on the molecule. With each triglyceride one or two positions will be occupied by one fatty acid while the other one or two positions will be occupied by one or two other fatty acids.

In glycerol



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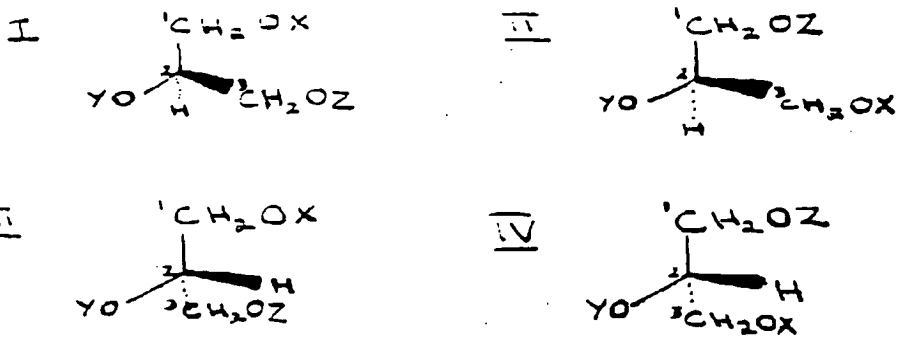
the 1 and 3 positions. while formally identical, in derivatives may not be functionally so. A triglyceride with three different fatty acids X, Y and Z is chiral at C_2 and four structures can notionally be drawn:-

Table 2



In solution, II is identical to III, which is the optical isomer of I, as can be seen by considering rotation of the molecule about the bond to YO- to bring -CH_2OX to the top, and the same is so of I and IV. However in biological systems where a receptor favours bonding groups in a given relative position, it may be preferable or even necessary to have one isomer or the other.

In preparing triglycerides

The course of esterification may in principle be directed to favour a desired isomer, but if it is not, then the position of individual acid residues in the triglycerides produced from starting mixtures of two acids is one of the several possibilities:-

Table 3a

AAA	AAB•
ABA	ABB•
BAB	
BBB	

and for three different acids:-

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Table 3b

AAA	AAB•	AAC•
ABA	ABB•	ABC•
ACA	ACB•	ACC•
	BAB	BAC•
	BBB	BBC•
	BCB	BCC•
		CAC
		CBC
		CCC

with either isomer equally likely to be formed where C_2 is chiral, at least in chemical as opposed to enzymatic synthesis. What the two or three acids are, of course, depends on the choices made from the possible acids. What the preparations of the isomers are is calculable, for undirected synthesis.

The Invention

In the light of the above the invention provides, as groups of isomers or singly, triglycerides containing:-

a) two residues of an acid selected from oleic acid and the following groups of the acids of table 1:-
   i) GLA and DLA
   ii) AA, adrenic acid, and the 22:5 n-6 acid
   iii) stearidonic acid and the 20:4 n-3 acid
   iv) EPA, the 22:5 n-3 acid, and DHA

and one residue of an acid selected differently therefrom; or

b) one residue of an acid selected from oleic acid and the acids of groups i) to iv) above, one residue of an acid selected differently therefrom, and one residue of an acid selected differently again therefrom;

with the proviso that where an acid has been selected from one group a subsequent selection is not from that same group.

The groups of isomers so defined comprise mixtures of positional and/or optical isomers, which may be in the proportions arising from directed or undirected synthesis, or in proportions arising from treatment of as-synthesised mixtures to enhance the proportion of particular isomers or groups of isomers. Further, according to the method of synthesis and degree of enhancement if any, varying amounts of triglycerides other than those defined may also be present.

The selection of desired groups of isomers may also be tabulated as below, with arbitrary reference numbers for the triglycerides (TGs), or rather possible groups of triglyceride isomers represented. For example TG1 is the possible di- Group (i) - mono- Group (iv) glycerides eg. the di - (gammalinolenoyl) - mono - (eicosapentanoyl) glycerides. The table is:-

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Table 4

TG	Oleic	Group 1	Group 2	Group 3	Group 4
1	-	2	-	-	1
2	-	2	1	-	-
3	1	2	-	-	-
4	-	2	-	1	-
5	-	1	-	-	2
6	-	1	2	-	-
7	2	1	-	-	-
8	-	1	-	2	-
9	-	1	1	-	1
10	1	1	-	-	1
11	-	1	-	1	1
12	1	1	1	-	-
13	-	1	1	1	-
14	1	1	-	1	-
15	-	-	1	-	2
16	1	-	-	-	2
17	-	-	-	1	2
18	-	-	2	-	1
19	2	-	-	-	1
20	-	-	-	2	1
21	1	-	1	-	1
22	-	-	1	1	1
23	1	-	-	1	1
24	1	-	2	-	-
25	-	-	2	1	-
26	2	-	1	-	-
27	-	-	1	2	-
28	1	-	1	1	-
29	2	-	-	1	-
30	1	-	-	2	-

As well as in structural terms as above, the invention may be considered in terms of starting mixtures of acids, selected from oleic acid and the acids of Groups i) - iv) above, namely in molar terms (66% stands for two thirds, 33% for one third):-

i) 66% of an acid selected from oleic acid and the acids of Groups i), ii), iii) and iv), and 33% of a different acid selected therefrom; or

ii) 33% of an acid selected from oleic acid and the acids of Groups i), ii), iii) and iv); 33% of a different acid selected therefrom; and 33% of another different acid selected therefrom.

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Preferred starting mixtures, with arbitrary reference numbers for the triglycerides, or rather possible groups of triglyceride isomers (TGs), that they formally represent, are derived from Table 4, specifying numbers of residues, by reading 66 mole % (two thirds) for '2' and 33 mole % (one third) for '1'.

As the desire is to give mixed triglycerides of two or three acids, species AAA and BBB of Tables 3a and 3b are for example unwanted components of the synthesized mixture, but the mixed species predominate and the as-synthesized mixtures are therefore valuable. Where the desire specifically is to give mixed triglycerides of three acids, such species do not predominate but are still present in a valuable proportion. In either case desired species can be separated or part separated from others by chromatographic or other methods known in themselves.

Individual triglycerides, either containing three different fatty acids, or two fatty acids in a 2:1 ratio, may thus be manufactured by chemical or enzymatic means by methods known in themselves to those skilled in the art. If the method of synthesis or manufacture does not provide an adequate concentration of the desired triglyceride, then that triglyceride may be concentrated and purified by appropriate techniques as outlined later.

As far as we are aware, all the groups of triglyceride isomers defined as above consist of new triglycerides which do not appear in nature and have not previously been described. They may broadly be prepared as follows:

a) The individual fatty acids are purified from natural animal, vegetable or microbial sources or are chemically synthesized, there being methods known in themselves to those skilled in the art.

b) The individual fatty acids are then esterified with glycerol by chemical or enzymatic methods, there being again methods known in themselves to those skilled in the art. For example, the fatty acids and glycerol may be allowed to react together in the presence of one of a number of appropriate enzymes, or of p-toluene sulphonic acid hydrate.

c) If required, the specific triglycerides are further purified by appropriate methods, again known to those skilled in the art, in particular high pressure liquid chromatography or other appropriate forms of chromatography; low temperature crystallisation; or the use of solvents which differentially select triglycerides of particular composition.

In the product, desirably a specified particular triglyceride or group of triglycerides forms more than 10%, preferably more than 30% very preferably more than 70% and ideally more than 90% of the triglycerides present in any triglyceride mixture used for the preparation of pharmaceutical compositions, foods, or skin care products. The triglycerides may be made up into appropriate pharmaceuticals or foods so as to provide a daily dose of 1mg to 100g per day, preferably 10mg to 10g and very preferably 500mg to 4g. Alternatively in foods or skin care products the triglycerides may be incorporated in concentrations of 0.001 to 50%, preferably 0.05 to 20% and very preferably 0.1 to 5%.

The specified triglycerides have a wide variety of possible uses. They may be used as pharmaceuticals for the treatment or prevention of disease in which abnormalities of EFAs have been identified. They may be added to foods or be added to or used as nutritional supplements for those who require the particular EFAs for the treatment or prevention of disease. They may also be used in foods or pharmaceuticals for veterinary use. They may be used for skin care.

The triglycerides may be formulated in any way appropriate, as well known to those skilled in the art of preparing pharmaceuticals, skin care products or foods. They may be administered orally, enterally, topically, parenterally, (subcutaneously, intramuscularly, intravenously or by any other route), rectally, vaginally or by any other appropriate route.

Synthetic Examples

The following are examples of synthesis of the triglycerides.

Fourteen triglycerides have been prepared as examples of the range of triglycerides outlined in Table 4, as summarised in Table 5:-

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Table 5

Code	Triglyceride	TG number (as in Table 2)
GGA	GLA/GLA/AA	1
GGO	GLA/GLA/OA	3
GGE	GLA/GLA/EPA	4
GGD	GLA/GLA/DHA	4
GAA	GLA/AA/AA	5
GOO	GLA/OA/OA	7
GEE	GLA/EPA/EPA	8
GED	GLA/EPA/DHA	8
GOA	GLA/OA/AA	10
GAE	GLA/AA/EPA	11
GAD	GLA/AA/DHA	11
GOD	GLA/OA/DHA	14
AED	AA/EPA/DHA	20
OED	OA/EPA/DHA	30

There are a large number of synthetic routes to triglycerides reported in the literature but we have been concentrating on two main methods. The first uses glycerol monoprotected with a 4-methoxybenzyl group as the starting point. At a later stage in the synthesis this group is removed using a boron reagent, but while this very efficiently removes the protecting group and minimises acyl group scrambling it also causes *cis-trans* isomerisation of the fatty acid double bonds. This fact coupled with the expense of the reagent severely limits the applicability of this route. The second main route starts with a base-catalysed epoxide ring opening of a glycidol by a fatty acid to yield a monoacylglycerol. This route does lead to a mixture of positional isomers but nevertheless has good potential for larger scale syntheses. Direct reaction between glycerol and a mixture of fatty acids mediated either by DCC/DMAP or p-toluenesulfonic acid has also been carried out using a mixture of two different fatty acids. Assuming that both fatty acids react at equal rates simple probability theory can be applied to predict the distribution of triglyceride products. For example when equal parts of 2 different fatty acids (A,B) are reacted with glycerol using p-toluenesulfonic acid catalysis. Four classes of triglyceride will be formed:

AAA	12.5%
AAB, ABA, BAA	37.5%
ABB, BAB, BBA	37.5%
BBB	12.5%

If the fatty acids are in a ratio of 2 parts A: 1 part B the theoretical preparations are:-

AAA	29.6%
AAB, ABA,BAA	44.4%
ABB,BAB,BBA	22.2%
BBB	3.7%

In actual measurements it should be noted that due to different extinction coefficients at 210nm (the monitoring wavelength used for the hplc analysis) the percentages measured by hplc are different to the theoretical values.

The same approach can be used to examine the distribution when 3 different fatty acids are used. when in reaction as above ten classes of triglyceride will be formed:

AAA	3.7%
BBB	3.7%
CCC	3.7%

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AAB, ABA, BAA	11.1%
AAC, ACA, CAA	11.1%
BBA, BAB, ABB	11.1%
BBC, BCB, CBC	11.1%
CCA, CAC, ACC	11.1%
CCB, CBC, BCC	11.1%
ABC, ACB, BAC, BCA, CAB, CBA	22.2%

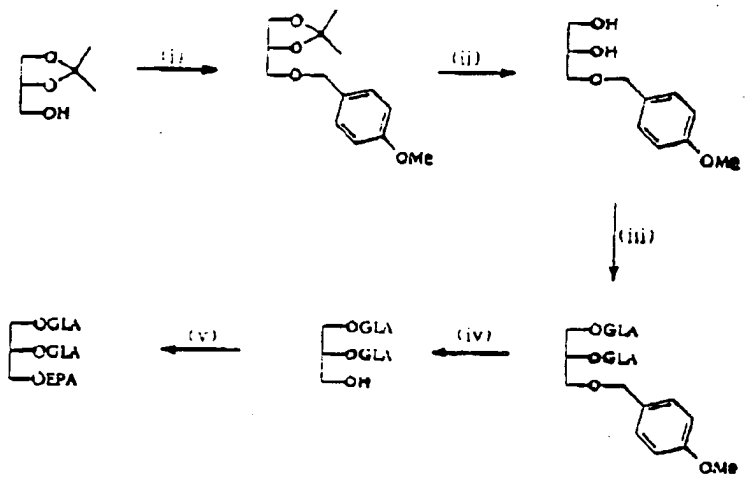
In the light of the large number of triglyceride products and a maximum yield of 22.2% a directed synthesis is preferred.

The invention is illustrated by the following preparative examples, in which the following abbreviations occur:-

DCC = dicyclohexylcarbodiimide
DMAP = 4-N,N-dimethylaminopyridine
OA = oleic acid (*cis*-9-octadecenoic acid)
GLA = γ-linolenic acid (*cis,cis,cis* - 6,9,12-octadecenoic acid)
AA = arachidonic acid (*cis,cis,cis,cis,cis*-5,8,11,14-eicosatetraenoic acid)
EPA = *cis,cis,cis,cis,cis*-5,8,11,14,17-eicosapentaenoic acid
DHA = *cis,cis,cis,cis,cis,cis,cis*, - 4,7,10,13,16,19-docosahexaenoic acid

Method A - used for the preparation of GGA, GGO,GGE, GGD, GAA, GOO an GEE.

Specific Example 1: preparation of GLA/GLA/EPA



(i) A mixture of solketal (3.3g, 25 mmol), tetrabutylammonium hydrogen sulfate (425mg, 1.25 mmol, 5 mol%), sodium hydroxide (6.0g, 150 mmol), 4-methoxybenzyl chloride (4.7g, 30 mmol), water (6ml) and *trans*-1,2-dichloroethene (20ml) was stirred vigorously under reflux until tlc (10% acetone/hexane) showed the reaction to be complete (typically 3-7 hours). On completion the reaction mixture was cooled and diluted with water (20ml) and methylene chloride (20ml). The organic layer was separated and washed with water until the washings were neutral (4x 30ml). The organic layer was dried ($MgSO_4$) and concentrated to dryness. Purification by flash chromatography (8% acetone/hexane) yielded the fully protected glycerol as a colourless oil.

(ii) A mixturex of the fully protected glycerol (*vide supra*) (1.0g), hydrochloric acid (1M, 10ml) and methanol (15ml) were stirred together at room temperature for 1h. (At this point tlc analysis (25% ethyl acetate/hexane) showed complete disapperance of the starting material and the formation of one new spot corresponding to the product). The bulk of the solvent was removed, brine (20ml) was added and the product was extracted into methylene chloride (4 x 30ml). The combined extracts were dried ($MgSO_4$) and concentrated to dryness. On standing under high vacuum the product crystallised. On one occasion it was purified by flash chromatography (3% methanol/methylene chloride) although this was not generally nec-

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essary. This monoprotected glycerol was the starting point for attachment of the fatty acids.
(iii) A solution of DCC (2.8g, 13.5 mmol) and DMAP (1.5g, 12.4mmol) in methylene chloride (20ml) was added to a solution of the monoprotected glycerol (1.15g, 5.4mmol) and GLA (95%, 3.5g, 12.4mmol) in methylene chloride (40ml) at room temperature under nitrogen. As the reaction proceeded a precipitate of dicyclohexylurea formed. After 2 h tlc analysis (25% ethyl acetate/hexane) indicated that the reaction was complete. Hexane (60ml) was added to precipitate more dicyclohexylurea and the reaction was filtered and concentrated to dryness. Purification by flash chromatography (25 % ethyl acetate/hexane) yielded the monoprotected diacylglycerol as a colourless oil.
(iv) Bromodimethylborane (85µl, 0.84 mmol) was added by syringe to a solution of the monoprotected diacylglycerol (310mg, 0.42 mmol) in methylene chloride (10ml) at -78°C (external cooling by dry ice/acetone) under nitrogen. After 3 minutes at -78°C the reaction was quenched by the addition of diethyl ether (100ml). Tlc analysis (4% acetone/chloroform) indicated that the reaction had gone substantially towards completion. The mixture was washed with water (5 x 100ml), brine (100ml), dried ($MgSO_4$) and concentrated to dryness. The product was used direcly in the next step without any further purification.
(v) A solution of DCC (120mg, 0.55 mmol) and DMAP (60mg, 0.48 mmol) in methylene chloride (5ml) 1was added to a solution of the crude diacylglycerol (0.42mmol) and EPA (98%, 145mg, 0.48mmol) in methylene chloride (10ml) at room temperature under nitrogen. As the reaction proceeded a precipitate of dicyclohexylurea formed. After 2 h tlc analysis (8 % ethyl acetate/hexane) indicated that the reaction was complete. Hexane (30ml) was added to precipitate more dicyclohexylurea and the reaction was filtered and concentrated to dryness. Purification by flash chromatography (5 % ethyl acetate/hexane) yielded the pure triglyceride as a colourless oil.

Method B - used for the preparation of GOA and GOD

Specific Example 2 - preparation of GLA/OA/AA
(i) as for Method A
(ii) as for Method A
(iii) A solution of DCC(535mg, 2.7mmol) and DMAP (335mg, 2.7mmol) in methylene chloride (10ml) was added dropwise to a cooled (0°C) solution of the monoprotected glycerol (500mg, 2.4mmol) and GLA (95%, 620mg, 2.2mmol) in methylene chloride (40ml) and the resulting solution was stirred at 0°C for 4h. During the course of the reaction a fine precipitate of dicyclohexylurea formed. After 4h, tlc analysis (25% ethyl acetate/hexane) indicated complete disappearance of GLA and the formation of 2 new spots corresponding to both positional isomers of monacylated monoprotected glycerol.

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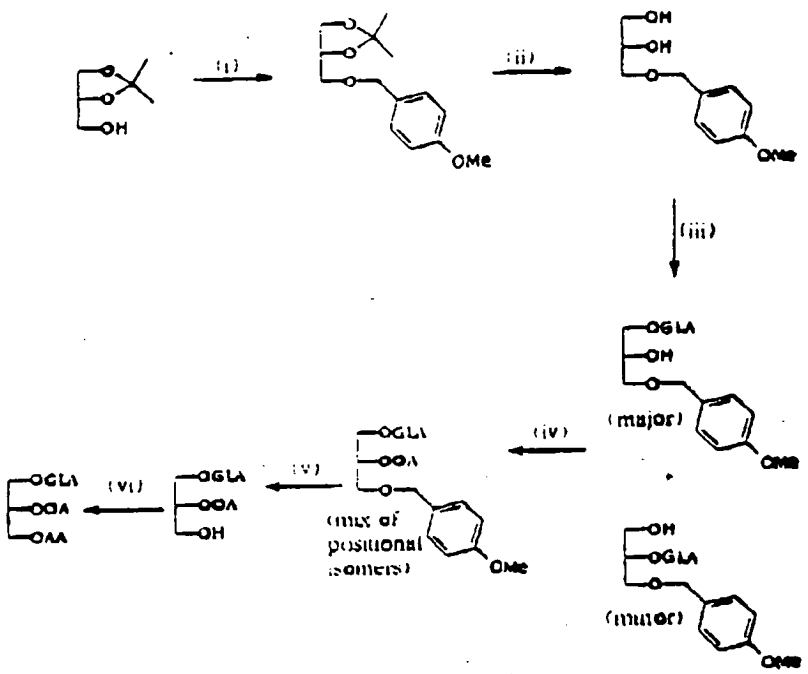

Hexane (50ml) was added to precipitate more dicyclohexylurea and the mixture was filtered, concentrated to dryness and purified by flash chromatography (25% ethyl acetate/hexane) yielding the product as a colourless oil.

(iv) A solution of DCC (230mg, 1.11 mmol) and DMAP (115mg, 0.93 mmol) in methylene chloride (5ml) was added to a solution of the mixture of monoacylglycerol isomers (400mg, 0.85mmol) and OA (99%, 270mg,) 0.93mmol) in methylene chloride (10ml) at room temperature under nitrogen. As the reaction proceeded a precipitate of dicyclohexylurea formed. After 3½h tlc analysis (25% ethyl acetate/hexane) showed the reaction to be complete. Hexane (30ml) was added to precipitate more dicyclohexylurea and the resulting mixture was filtered, concentrated to dryness and purified by flash chromatography (8 % ethyl acetate/hexane) to yield the product as a colourless oil.

(v) Bromodimethylborane (85µl, 0.84 mmol) was added by syringe to a solution of the monoprotected diacylglycerol (300mg, 0.41 mmol) in methylene chloride (10ml) at -78°C (external cooling by dry ice/acetone) under nitrogen. After 3 minutes at -78°C the reaction was quenched by the addition of diethyl ether (100ml). Tlc analysis (4% acetone/chloroform) indicated that the reaction had gone substantially towards completion. The mixture was washed with water (5 x 100ml), brine (100ml), dried ($MgSO_4$) and concentrated to dryness. The product was used directly in the next step without any further purification.

(vi) A solution of DCC (280mg, 1.37 mmol) and DMAP (150mg, 1.21mmol) in methylene chloride (5ml) was added to a solution of the crude diacylglycerol (1.05mmol) and AA (95%, 370mg, 1.21mmol) in methylene chloride (15ml) at room temperature under nitrogen. As the reaction proceeded a precipitate of dicyclohexylurea formed. After 2 h tlc analysis (8% ethyl acetate/hexane) indicated that the reaction was complete. Hexane (30ml) was added to precipitate more dicyclohexylurea and the reaction was filtered and concentrated to dryness. Purification by flash chromatography (5 % ethyl acetate/hexane) yielded the pure triglyceride as a colourless oil.

Method C - preparation of GED, GAE, GAD, AED and OED

Specific Example 3 - preparation of GED

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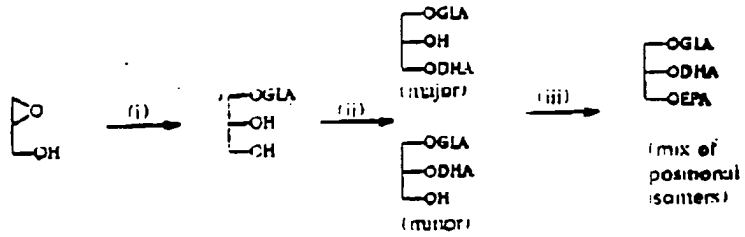

(i) A mixture of GLA (97%, 1.0g, 3.6mmol), glycidol (280mg, 3.73 mmol) and tri-n-butylamine (20µl, 0.08mmol) was heated at 85°C under nitrogen for 5h. The reaction was then cooled an purified by flash chromatography (5% methanol/methylene chloride) to yield the monoacylglycerol as a colourless oil.
(ii) A solution of DCC (270mg, 1.31mmol) and DMAP (160mg, 1.31mmol) in methylene chloride (5ml) was added to a solution of the monoacyiglycerol (400mg, 1.14mmol) and DHA (98%, 350mg, 1.08mmol) in methylene chloride (15ml) at 0°C under nitrogen. Tlc analysis (3% methanol/methylene chloride) after 5h showed the reaction to be complete. Hexane (30ml) was added to precipitate dicyclohextyurea and the mixture was filtered, concentrated to dryness and purified by flash chromatography (2% methanol/methlene chloride) to yield a mixture of diacylglycerol positional isomers as a colourless oil.
(iii) A solution of DCC (80mg, 0.39 mmol) and DMAP (40mg, 0.35mmol) in methylene chloride (5ml) was added to a solution of the diacylglycerol (200mg, 0.3mmol) and EPA (98%, 110mg, 0.35mmol) in methylene chloride (10ml) at room temperature under nitrogen. As the reaction proceeded a precipitate of dicyclohexylurea formed. After 2 h tlc analysis (8% ethyl acetate/hexane) indicated that the reaction was complete. Hexane (30ml) was added to precipitate more dicyclohexylurea and the reaction was filtered and concentrated to dryness. Purification by flash chromatography (5% ethyl acetate/hexane) yielded the pure triglyceride as a colourless oil.

Method D - Specific example by, preparation of GGO and GOO as a mixture (using a 2:1 ratio of GLA: OA).

A solution of DCC (725mg, 3.5mmol) and DMAP (430mg, 3.5mmol) in methylene chloride (10ml) was added to a solution of glycerol (200mg, 2.2mmol), GLA (95%, 610mg, 2.2mmol) and OA (99%, 310mg, 1.1mmol) in methylene chloride (40ml) at room temperature under nitrogen. As the reaction proceeded a precipitate dicyclohexylurea formed. After 5h hexane (50ml) was added to precipitate more dicyclohexylurea and the reaction was filtered and concentrated to dryness. Purification by flash chromatography (5% ethyl acetate/hexane) yielded the pure triglycerides as a colourless oil.

Method E - Specific exmaple 5, preparation of GGO and GOO as a mixture using a 1:2 ratio of GLA:OA).

A solution of DCC (725mg, 3.5mmol) and DMAP (430mg, 3.5mmol) in methylene chloride (10ml) was added to a solution of glycerol (200mg, 2.2mmol), GLA (98%, 305mg, 1.1mmol) and OA (99%, 620mg, 2.2mmol) in methylene chloride (40ml) at room temperature under nitrogen. As the reaction proceeded a precipitate dicyclohexylurea formed. After 5h hexane (50ml) was added to precipitate more dicyclohexylurea and the reaction was filtered and concentrated to dryness. Purification by flash chromatography (5% ethyl acetate/hexane) yielded the pure triglycerides as a colourless oil.

Method F - Specific example 6, preparation of GGO and GOO as a mixture (using a 2:1 ratio of GLA:OA)

A mixture of glycerol (200mg, 2.2mmol), GLA (98%, 610mg, 2.2mmol), OA (99%, 305mmol, 1.1mmol) and p-toluenesulfonic acid (20mg) were heated at 140°C for 5 h under a stream of nitrogen. The reaction was cooled and purified by flash chromatography (5 % ethyl acetate/hexane) to yield the pure triglycerides as a colourless oil.

Method G - Specific Example 7 preparation of GGO and GOO as a mixture (using a 1:2 ratio of GLA:OA).

A mixture of glycerol (200mg, 2.2mmol), GLA (98%, 305mg, 1.1mmol), OA (99%, 620mmol, 2.2mmol) and p-toluenesulfonic acid (20mg) were heated at 140°C for 5 h under a stream of nitrogen. The reaction was cooled and purified by flash chromatography (5 % ethyl acetate/hexane) to yield the pure triglycerides as a colourless

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oil.

The products of the above examples have been subject to hplc triglyceride analysis (Table 6) and gc fatty acid analysis (Table 7), by standard methods.

In gc analysis the preparation of the methyl ester derivatives of fatty acids is well known. Boron trifluoride in methanol (12-14% w/v) was used as catalyst. 100mg of each of the triglycerides was transesterfied and analysed in a Hewlett Packard 5890 Series II equipped with a Supelcowax™ 10 capillary column (30m x 0.53mm x 1.0μm). Injector temperature set at 220°C and detector temperature at 250°C, the oven temperature was programmed starting at 180°C for 5 min after which is increased at a rate of 2°C/min until 210°C was reached and maintained at this temperature for a further 15 mins. 1μl of each of the methyl esters of the fatty acids was injected using an autosampler, 7673 from Hewlett Packard. Each of the methyl esters of the fatty acids were identified by injecting standards.

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Table 6

Example	Triglyceride	Retention Time	Area % (peaks over 2.5%)
1	GGA	11.21	83.632
		13.73	10.320
	GGO	17.32	82.987
		21.76	11.013
	GGE	9.00	84.844
		10.69	3.452
		10.89	5.031
	GGD	9.29	86.471
		11.22	9.976
	GAA	11.15	91.523
		13.62	5.097
	GOO	3.28	4.434
		26.87	89.002
	GEE	7.59	89.576
		9.08	2.982
2	GOA	16.31	9.243
		17.77	82.816
		22.31	3.018
	GOD	15.35	91.699
		19.16	4.812
3	GED	6.798	90.502
		8.030	6.201
	GAE	7.977	91.212
	GAD	8.198	92.140
		9.802	4.202
	AED	6.949	92.234
	OED	10.132	98.334

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(Table 6 cont'd)

Example	Triglyceride	Retention Time	Area % (peaks over 2.5%)
4	GGO/GOO	10.67	31.838 (GGG)
		13.09	9.143
		17.30	44.518 (GGO)
		21.75	3.660
		29.49	6.952 (GOO)
5	GGO/GOO	8.711	9.270 (GGG)
		13.423	33.716 (GGO)
		21.423	35.741 (GOO)
		21.942	11.338
		35.684	4.651
6	GGO/GOO	8.947	35.266 (GGG)
		13.996	42.886 (GGO)
		22.974	13.228 (GOO)
7	GGO/GOO	8.915	27.091 (GGG)
		13.943	38.959 (GGO)
		22.730	22.455 (GOO)

Table 7

Example	Triglyceride	Retention Time	Area % (peaks over 2.5%)
1	GGA	7.879	4.1081 (LA)
		8.568	58.6674 (GLA)
		14.140	35.2856 (AA)
	GGO	6.954	33.7482 (OA)
		7.896	3.8946 (LA)
		8.629	60.6707 (GLA)
	GGE	8.589	61.1291 (GLA)
		9.108	3.5780
		16.017	33.0809 (EPA)

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(Table 7 cont'd)

Example	Triglyceride	Retention Time	Area % (peaks over 2.5%)
	GGD	7.830	3.4747 (LA)
		8.576	55.9686 (GLA)
		9.047	3.1251
		23.250	34.7093 (DHA)
	GAA	8.577	30.6452 (GLA)
		14.168	65.1502 (AA)
	GOO	6.969	65.1579 (OA)
		8.570	31.6086 (GLA)
	GEE	8.583	31.4162 (GLA)
		16.072	63.7405 (EPA)
2	GOA	6.936	30.8227 (OA)
		8.576	27.4197 (GLA)
		14.159	36.1341 (AA)
	GOD	6.949	31.9633 (OA)
		8.602	29.8198 (GLA)
		23.343	34.8294 (DHA)
3	GED	8.599	29.2576 (GLA)
		16.056	32.4455 (EPA)
		23.353	35.4802 (DHA)
	GAE	8.591	31.3688 (GLA)
		14.173	32.2683 (AA)
		16.043	32.9175 (EPA)
	GAD	8.600	29.8217 (GLA)
		14.187	31.2864 (AA)
		23.350	35.0575 (DHA)
	AED	14.176	30.3248 (AA)
		16.048	31.5008 (EPA)
		23.333	35.1120 (DHA)
	OED	6.956	31.4497 (OA)
		16.063	32.3986 (EPA)
		23.354	35.1133 (DHA)

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Use Examples.

The following are examples of modes of use of the triglycerides.

1. Any one or any mixture of the triglycerides specified in Table 2 made up in soft or hard gelatin capsules of any size between 100mg and 1g and administered to provide a daily dose of between 100mg and 10g.
2. Any one or any mixture of the specified triglycerides microencapsulated in gelatin or agar or any other appropriate material, or incorporated into any appropriate material to form a powder which can be taken orally, added to foods, tabletted, encapsulated, packed in sachets or any other appropriate form.
3. Any one or more of the specified triglycerides made up in a whip, liquid, cream or other appropriate form for oral administration.
4. Any one or more of the specified triglycerides made into a cream, ointment or other topical preparation at a concentration ranging from 0.1 to 30%.
5. Any one or more of the specified triglycerides made up into an emulsion suitable for parenteral administration.
6. Any one or more of the specified triglycerides added to any appropriate food material such as a spread, drink, candy, cereal, infant food or bakery product.

Claims

1. As groups of isomers or singly, triglycerides containing:-
   a) two residues of an acid selected from oleic acid and the following groups of the acids of table 1 herein:-
      i) GLA and DGLA
      ii) AA; adrenic acid, and the 22:5 n-6 acid
      iii) stearidonic acid and the 20:4 n-3 acid
      iv) EPA, the 22:5 n-3 acid, and DHA and one residue of an acid selected differently therefrom; or
   b) one residue of an acid selected from oleic acid and the acids of groups i) to iv) above, one residue of an acid selected differently therefrom, and one residue of an acid selected differently again therefrom;
   with the proviso that where an acid has been selected from one group a subsequent selection is not from that same group.

2. Triglycerides according to claim 1, containing acid residues selected as set out in Table 4 herein under any one of the references TG1 to TG30.

3. Triglycerides according to claim 1 or 2, in the form of mixtures with other triglycerides, made by reaction of acid and glycerol starting materials in kinds and proportions corresponding to the selected residues.

4. Triglycerides according to claim 3, wherein the triglycerides according to claim 1 or 2 constitute more than 10 preferably more than 30 very preferably more than 70 and ideally more than 90 molar percent of the mixtures.

5. Triglycerides according to any preceding claim in the form of a pharmaceutical composition generally.

6. Triglycerides according to any of claimsl to 4 in the form of a nutritional supplement or food composition.

7. Triglycerides according to any of claims 1 to 4 in the form of a topical pharmaceutical, skin care or cosmetic composition.

8. Triglycerides according to claims 5, 6 or 7 wherein the composition is in a form to provide a daily dose of 1mg to 100g preferably 10mg to 10g and very preferably 500mg to 4g of triglyceride according to claim 1 or 2.

9. Triglycerides according to claims 5, 6 or 7 wherein the composition comprises 0.001 to 50% preferably 0.05 to 20% and very preferably 0.1 to 5% by weight of triglyceride according to claim 1 or 2.

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